Antibacterial Behavior of Synthesized Silica-Silver Nanocomposite for Drinking Water Purification

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Abstract

As worldwide population growth and climate change, important challenges in the global water circumstances require novel water technologies to the supply of drinking water. Nanocomposites are also increasingly used to improve water quality. In this research area, our aim is to apply the water purification based on the distinctive properties of silica-silver nanocomposite. In the synthesis of Silica-silver nanocomposites, nanosilica was synthesized by chemical modification method and MPTMS was used as a linker to combine the silica and silver nanoparticles. The synthesized materials were characterized by using powder X-ray diffraction (XRD) analysis, and scanning electron microscopy (SEM) and size distribution analysis (Zeta sizer). The antibacterial activity of synthesized nanocomposites was tested by agar well diffusion method and test tube method. These nanocomposites are being painted into ceramic filters for their antimicrobial properties during water treatment. The contaminated water samples that may have waterborne pathogenic bacteria from Hlaing River and Ngamoeyeik creek were collected and passed through the nanocomposite painting ceramic filter. *E.coli*/ coliform petrifilms were used to determine the bactericidal activity by comparing the concentrations of target organisms before and after passing through these nancomposite filters. The result showed that SiAg nanocomposite ceramic filter have higher bactericidal activity than colloidal silver. It is seen that Silica matrix enables incorporation of metal ions silver in low concentrations and will provide the long-lasting antibacterial activity as their gradual release into the environment.

Keywords: silica-silver nanocomposite, bactericidal activity, petrifilms, ceramic filter

1. Introduction

Water is essential to life. An adequate, safe and accessible supply must be available to all. Improving access to safe drinking-water can result in significant benefits to health (WHO,2008). However, rapid industrialization is continuously degrading the quality of water due to addition of large amounts of pollutants into the water (M. A. Shannon et al., 2008, X. Qu, P. J. Alvarez and Q. Li, 2013). The consumption of drinking water contaminated with human and animal excreta is the greatest risk for infection from microbes in water. The non-pathogenic organisms that are always present in the intestines of humans and animals are excreted along with the pathogens, but in far greater numbers. Several of these coliforms are easily isolated and are ideal for use as indicators of fecal contamination. *Escherichia coli*, a member of the coliform group, can survive for several weeks under ideal conditions and are far more easily detectable than the other indicator bacteria. Water pollutants have appeared as threats to entire biosphere, so their removal has become essential. The fundamental requirements for water purification are appropriate materials with high separation capacity, low cost, porosity, and reusability (P. Malaviya and A. Singh., 2011, A. Bhatnagar and M. Sillanpää., 2009). Every effort should be made to achieve a drinking water quality as safe as possible. In this regard, nanotechnology provides, an opportunity to develop advanced materials for effective water purification by optimizing their properties like hydrophilicity, hydrophobicity, porosity, mechanical strength, and dispersibility (Daer, J.et al., 2015). Nano particles having high surface area, can contribute a lot in water purification but its agglomeration restricts its use (P. Z. Ray and H. J. Shipley., 2015). However, agglomeration can be minimized by converting nanomaterials to nanocomposites. In this research area,

emphasis has been given on nanocomposite, coating in ceramic water filter and their use for water purification.

Nanoparticles, such as silver, TiO_2, and zinc oxide (ZnO), have anti-bacterial activity for drinking water treatment. Antimicrobial properties of silver have been known for centuries. Silver and its compounds have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities for bacteria, fungi, and virus since ancient times (Lok C N. et al., 2006). Compared with other metals, silver exhibits higher toxicity to microorganisms while it exhibits lower toxicity to mammalian cells (Zhao GJ and Stevens SE., 1998). Indeed, Ag+ binds to thiol groups (-SH) in enzymes leading to their deactivation, with several important outcomes, especially in the mitochondrial energy metabolism. Silver ions can also bind to DNA, increasing the stability of the double helix and denaturing the DNA molecule. Although, only the ionized form of silver is responsible for the antimicrobial action (Lok C N. et al., 2007). Lately, the recent advances in researches on metal nanoparticles appear to revive the use of silver nanoparticles (SNPs) for antimicrobial applications. SNPs have been applied to a wide range of healthcare products, such as burn dressings, scaffold, water purification systems, and medical devices (Thomas V. et al., 2007). The silver nanocomposite also very efficiently removes dyes and pathogenic microorganisms from water bodies in a single-step operation.

Nanosilica has a very high surface to volume ratio and contains a large number of surface hydroxyl groups, which provide electrostatic binding energy for dye molecules on its surface. Combining this with the antibacterial activity of the silver nanoparticles on the surface of nano-silica results in a synergistic effect of the nanocomposite, which is responsible for high removal of dyes and microorganisms from contaminated water.

A possible solution to tackle water purification problems has been exhibited by a group of researchers in India who created nanotechnology-based water purification using nano silica silver composite material as antimicrobial, antifouling and dye-adsorptive substance. Using this procedure, pathogenic bacteria and dye present in contaminated water can be treated concurrently without using any chemicals, electricity or high temperature (S. K. Das and A.B. Mandal et.,2013). Hollow silica nanospheres and nanotubes were synthesized as hosts for the immobilization of silver. It was observed that both composites had excellent antibacterial performance but Ag-supported tubular hollow structure showed a stronger antibacterial ability than spherical hollow structure because they can retain higher silver contents as well as smaller and more dispersed silver nanoparticles (Wang J-X. et al., 2016). Silver nanoparticles were immobilized onto the surface of magnetic silica composite to prepare magnetic disinfectant that exhibited enhanced stability and antibacterial activity. The silica coat not only acted as a supporting matrix, but also enhanced the stability of the disinfectant (D. E. Camporotondia and M. L. Fogliaa, 2013).

Cost-effective filter materials coated with Silver nanoparticles is an alternative technology. Ceramic water filters are an appealing water treatment technology. The silver acts as a well-studied antimicrobial agent without changing the taste, color, or odor of treated water. In this research study, silica-silver nanocomposite that has gradual release of silver to the environment and will provide a long-lasting antibacterial activity, was synthesized and applied to ceramic water filter for purified drinking water. The antibacterial activity of silica-silver nanocomposite on the test water before and after passing ceramic filter was evaluated by using *E.coli*/coliform petrifilm.

2. Material and Methods

2.1. Chemicals and Materials

TEOS (Tetra Ethyl Ortho Silicate) and 3-mercaptopropyl trimethoxysilane (MPTMS) were purchased from Sigma-Aldrich. Silver Nitrate, Colloidal silver, CTAB (Cetyltrimethyl Ammonium Bromide), Nitric acid and ethanol used in our experiments were of analytical grade. Muller-Hinton Agar, Nutrient Broth, E.M.B Agar and Plate count Agar were purchased from HIMEDIA, India. Deionized water was used throughout the experiments except for in the antibacterial tests, when sterilized H2O was used. All experiments were conducted in high-grade glassware. Ceramic water filter pots (CWP) supplied by Thirst aid association were used as received (pore size, 0.2 μm). Test water were collected from the Hlaing River,

Ngamoeyeik Creek and Hlawgar water distribution pipe-line to test the efficacy of the water treatment with environmental samples.

2.2. Methods

2.2.1. Synthesis of Si-Ag nanocomposites

Silica-silver nano-composite powders with 5 mol% silver was prepared by modified Stöber method. The precursors consisted of tetraethyl orthosilicate (TEOS), Cetyltrimethyl Ammonium Bromide (CTAB), 3-mercaptopropyl trimethoxysilane (MPTMS), silver nitrate, ethanol (C_2H_5OH), nitric acid (HNO_3) and distilled water. The molar ratio of TEOS: C_2H_5OH: HNO_3: CTAB: H_2O was 1: 4: 0.15: 0.125:10, respectively. Firstly, $AgNO_3$ and distilled water were mixed and stirred for 30 min at room temperature to form A-solution. In another beaker, TEOS, CTAB and ethanol were also mixed for 30 minutes to obtain B-solution. Then, B-solution was slowly poured into A-solution under stirring. After 30 min stirring, this mixture was added by HNO_3, ethanol and H_2O. After stirring, 10µl of MPTMS was added and the mixed solution was ultrasonicated for 1 hour. The final mixed solution was continuously stirred for 4 hours at room temperature and then placed into sealed polypropylene containers. After one-week sealing, the solution was gelled in oven at 60°C. The obtained bulk samples were grinded into fine powders. These powders were calcined in a furnace at 550˚C for 3 hr to decompose CTAB and to produce SiAg Nanocomposite. Another SiAg sample was also prepared without using MPTMS for comparisons.

2.2.2. Characterization of Synthesized Nanocomposite

The crystal structure of synthesized nanocomposite was characterized by x-ray diffraction (XRD) on D8 Advance Brucker, Germany. The morphology and structure of the prepared samples were characterized using field emission scanning electron micro-scope (FESEM; Auriga, Germany) attached to an energy-dispersive X-ray spectroscopy detector (EDX). The presence of silver in SiAg was also assessed by energy dispersive x-ray spectro-scopy (EDX). The UV-visible spectra were recorded over the range of 300–800 nm using the PerkinEler UV/VIS/NIR Lamda 950 spectrophotometer. The content of AgNPs in SiAg was determined by atomic absorption spectroscopy on a Zeenit 700P, Germany.

2.2.3. Evaluation of Antibacterial Activity

The antibacterial activities of SiAg nanocomposites were measured by two methods: Agar Well diffusion method and Test Tube Test. *Escherichia coli* (*E. coli*) was selected as an indicator of waterborne bacterial pathogen.

2.2.3.1. Test Tube Test

In vitro test, the bactericidal activity of synthesized nanocomposites was examined by test tube test. *E. coli* strains were cultured on Nutrient agar plates for 18hrs at 37 °C before use. 0.02g of SiAg powder, Nano Silica powder, $AgNO_3$ and colloidal silver were prepared by stirring the tested samples vigorously in ultrapure deionized water followed by 30-min sonication (FS30H, Fisher Scietific, 100W, 42 kHz) in a final volume of 50 ml. A single colony of *E. coli* was used for inoculating the NB medium. 0.025 ml of∼108 CFU mL −1 *E. coli* suspension was separately added to 50 ml of Nutrient Broth medium in 6 conical flasks (250ml). The cultures were shaken at 100 rpm for 1hr at 37°C under aerobic conditions. Then, 0.02g of each sample were introduced into tested flask. Suspensions of carriers ($AgNO_3$, silica nanoparticles, Collodial Silver and Amoxicillin) were used as controls. One flask without any samples is used as positive control containing only bacteria. All flasks were shaken again at 100 rpm for 1hr under aerobic conditions, and then *E. coli* suspensions were diluted tenfold in distilled water. 0.025 ml of each diluted solution was spread on plate count agar plates and then incubated at 37 °C overnight (∼16 h). At the end of the incubation period, the plates were evaluated for the presence or absence of growth. If the bacterial growth was found, the numbers of colonies on the plates are counted.

2.2.3.2. Agar Well diffusion Method

Agar well diffusion method was used to evaluate to the susceptibility pattern of *E. coli* microorganisms against SiAg nanocomposite material. Meullar Hinton Broth was prepared according to manufacturer's instruction and autoclaved at 121°C for 15 min. Briefly, 20 ml of Mueller Hinton agar was poured into an 80 mm-petri dish. After cooling the medium, the plates were then inoculated with 108 CFU/ml of bacteria. By punching the agar container with a sterile corkborer and scooping out the punched part, agar well of 8 mm diameter were made. The SiAg nanocomposite suspension at concentration of 2mg/ 25µl (0.2 M) was placed in each well. Suspensions of silica nanoparticles (0.2M), colloidal silver (0.2M), $AgNO_3$ (0.2M) and Amoxicillin (8µg/25µl) were used as control. The plates were left to stay for an hour to facilitate the diffusion of the solution. Then, the plates were incubated at 37 °C for 24 h. After 24 h of incubation, the diameters of the inhibition zones were measured.

2.2.4. Preparation of Ceramic Water Filter with Silica-Silver NCs for water purification

Firstly, 46 mg of silica-silver NCs powder was dissolved in 200ml of deionized water and the mixture was heated and ultrasonicated at 70°C to get well-dispersion. At the same time, 23 mg of SiAg NCs powder was mixed with 100ml of deionized water and ultrasonicated at 70°C. After 6h of ultrasonication, the sample was left for cooling. Then, the 200ml and 100ml of NCS solution are painted inside and outside of ceramic water filter separately by brushing and then allow drying. A higher volume is applied to the inside because there is more water in contact with this surface. Additionally, as water passes through, it will impregnate the nanocomposite deeper into the ceramic wall. The application on the outside of the pot also helps to prevent pathogens growing on the outside of the filter wall (Judy hagan and et.al) After painting and drying the ceramic water filter, these filters are need to adapt 30 L of water and the first filter water was examined for silver elution by AAS method.

Fig. 1. SiAg Coating in ceramic water filter

2.2.5. Evaluation of Antibactericidal activity of Synthesized NCs coated Filter

The bacterial removal efficiency was obtained by comparing the concentrations of target organisms before and after treatment. Bacterial contaminated water samples were collected from Ngamoeyate creek, Hlaing River and Hlawgar tap water in order to evaluate the antibactericidal activity. *E. coli* and total coliforms bacteria were examined by 3M Petri-films plates.1ml of after and before filtering water samples were added to petri-films and incubated at 37 °C overnight (~24 h). After incubation, the numbers of red and blue colonies were counted. The antibacterial efficiency of filtered water samples was calculated according to equation.

$$\eta(\%) = (N_o - N) \times 100/N_o$$

Where No and N are survival number of bacteria in after and before filtered water samples, respectively.

3. Results and discussion

The synthesis of the silica-silver nanocomposite (SiAg NCs), silver nanoparticles into the silica matrix by modified chemical reduction, was characterized by X-ray diffractometer. XRD pattern for synthesized SiAg NCs AgNPs without linker, MPTMS was shown in Figure 2. The broadened XRD peak for amorphous silica was centered at 2θ values (22.6). This pattern also shows the peaks at 2θ values (44.34, 64.50,74.43) indicating the presence of silver embedded in silica matrix. Also, peaks are observed at 2 θ values (54.70 and 31.93) may correspond to presence of silver oxide nanoparticles.

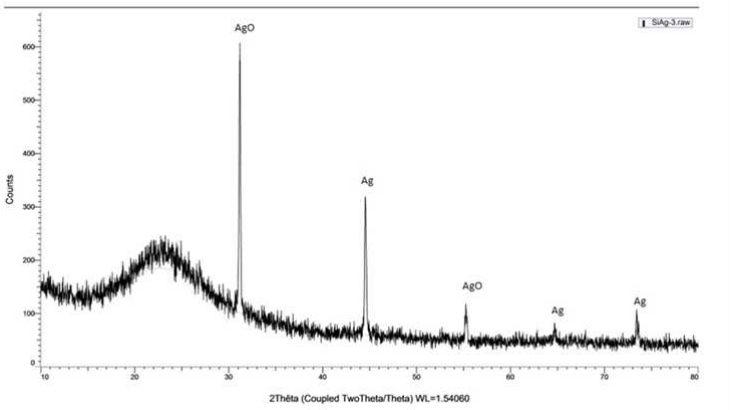

Fig. 2. XRD pattern of synthesized SiAg NCs without linker, MPTMS (SiAg)

XRD pattern for synthesized SiAg NCs AgNPs using with MPTMS as linker was shown in Figure 3. The broadened XRD peak for amorphous silica was centered at 2θ values (15). This peak was shifted to lower 2θ values by increasing the heating temperature. This pattern also shows four main characteristic diffraction peaks indicating face-centered cubic Ag crystals (JCPDS no. 00-004-0783) and indicating the presence of silver embedded in silica matrix.

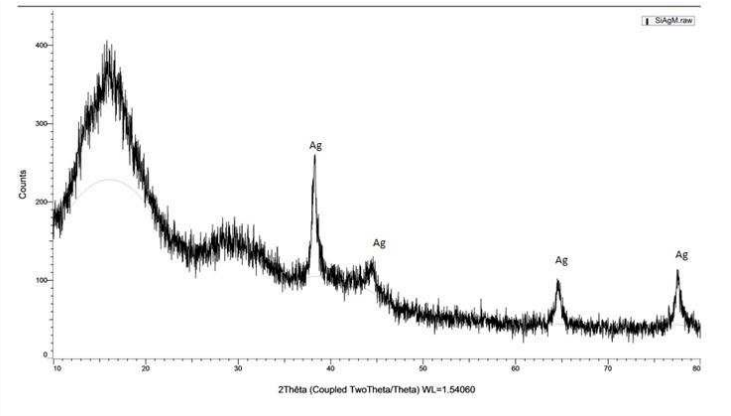

Fig.3. XRD pattern of synthesized SiAg NCs with linker, MPTMS (SiAg-M)

The SEM image for SiAg NCs shows in Figure 4. SEM indicates that there may be some agglomeration in some cases. Ag nano-particles organize themselves in extended three-dimensional amorphous silica networks. The silica-silver core-shell figure was difficult. The attachment of silver to silica

matrix cannot be seen sharply in SEM image but the result of EDX and AAS, the silver nanoparticles were attached to the silica nanoparticles. Figure 5 show the result of EDX.

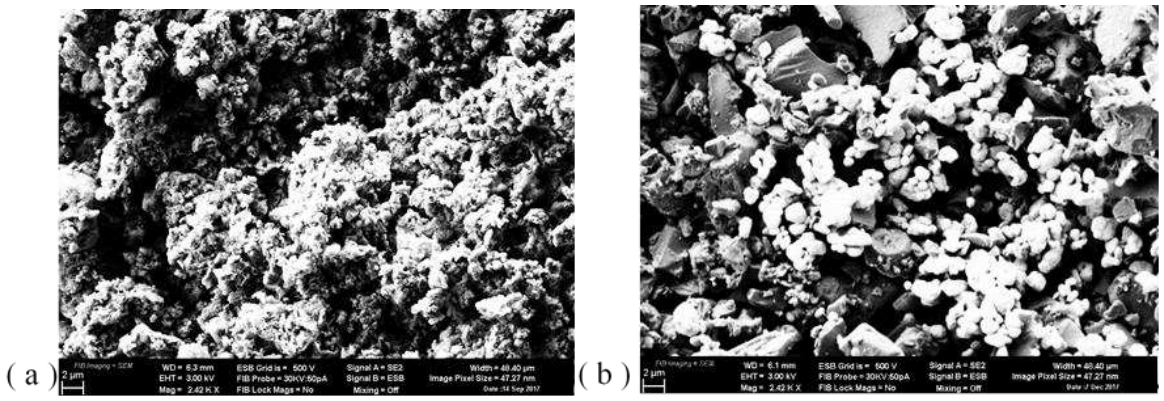

Fig. 4. SEM of (a) SiAg and (b) SiAg- M

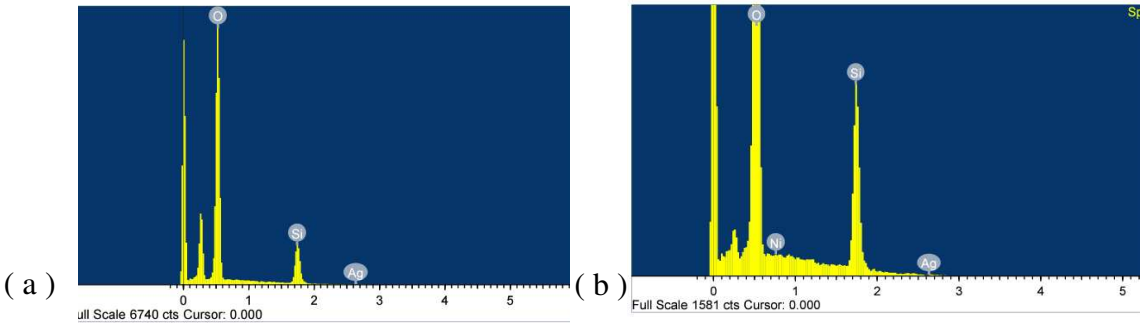

Fig. 5. EDX of (a) SiAg and (b) SiAg- M

Figure (6) showed the maximum absorption wavelength (λ max) of SiAg NCs at 408 nm. The symmetrical and sharp peak also could be observed, which indicates that the Ag NPs were relatively uniform distribution on these nanocomposites.

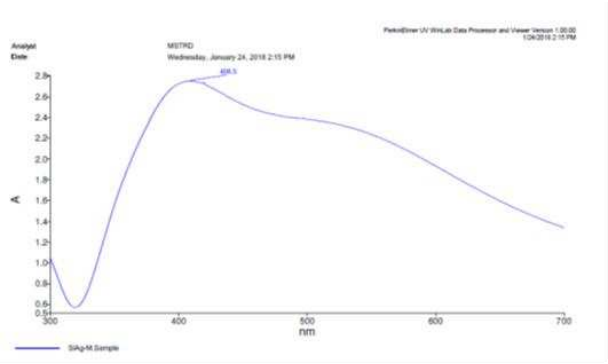

Fig. 6. UV-vis spectrum of SiAg

The amounts of silver in the nanocomposite were determined by atomic absorption spectrometry (AAS). The results revealed that the amounts of silver were 528mg/ kg.

The surviving number of *E. coli* in the tested medium was of zero for SiAg NCs, SiAgM, Collodial silver and Amoxicillin, all as shown in Table (1) and figure (7). Bacteria lawns are found in the plate of the bacterial control without any sample. The test tube test results indicated that SiAg sample exhibited highly bactericidal activity against *E. coli*.

Table 1. Antibacterial Activity of SiAg, SiAg M and Bacterial Control By Test Tube Method

Samples	Bacterial Control	Nano Silica	Collodial Silver	Amoxicillin	$AgNO_3$	SiAg	SiAgM
Colony Count	Growth	Growth	No Growth	No Growth	No Growth	No Growth	No Growth
Amount (g/ml)	-	0.2/10	320ug	320ug	0.02/10	0.02/10	0.02/10

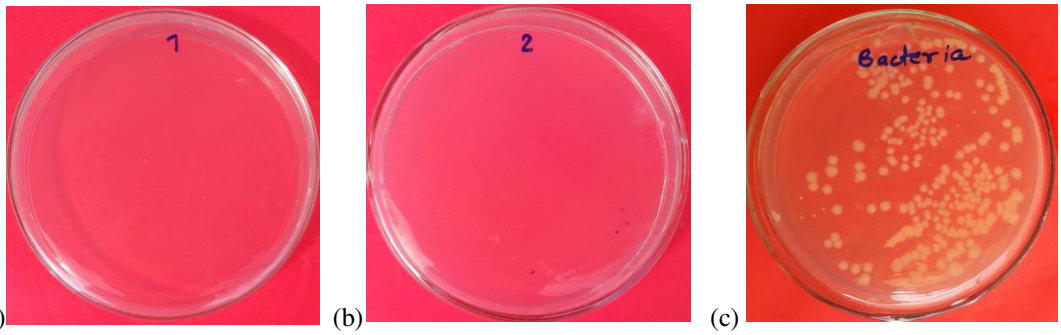

Fig. 7. Antibacterial activity of (a) SiAg, (b) SiAg M and (c) Bacterial control By Test tube method

Inhibition zone values were obtained by well diffusion method for the synthesized SiAg NCs and SiAg M tested against *E. coli.* The results are presented as zone diameter values in Table (2) and as shown in Figure (8). The AgNO3 collodial silver, Amoxicillin and SiAg NCs materials showed antibacterial activity against *E. coli*. SiAg has more distinct inhibition zones compared to SiAgM. According to WHO guidelines, SiAg can be classified as having moderate activity due to its inhibition zone of 12 mm. E. coli microorganisms were inhibited and killed at >2.0 mg of SiAg NCs materials. According to antimicrobial test by well diffusion and test tube method, SiAg and SiAg M have an antibacterial activity and similar as colloidal silver.

Table 2. Antibacterial Activity of SiAg, SiAg M and Bacterial Control By Well Diffusion Method

Sample	Amoxicillin (+) control	Nano Silica	Collodial Silver	SiAg (1)	SiAg (1)	SiAgM (2)	SiAgM (2)	Blank
Clear Zone Diameter (mm)	18-20	ND	11-12	12	12	11	10	ND

According to the test tube test and the Well diffusion test, SiAg, which showed moderate antibacterial activity, was applied to ceramic water filter and tested for evaluation of the antibactericidal activity to purify water. The silver content in the water samples was measured before and after filtration

through the ceramic water filter. The measured silver content in the water samples is shown in Table (3). After filtration of ceramic water filter coated with SiAg nanocomposites, silver content was non-detected in filtrated water by the results of AAS Method.

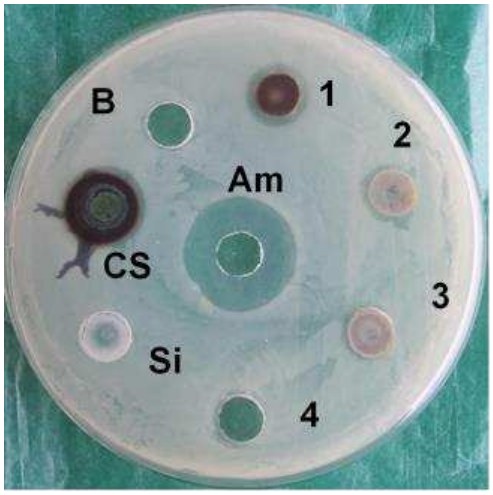

Fig. 8. Antibacterial activity of SiAg, SiAgM, Colliodial Silver by Agar Well diffusion Method

Table 3. Silver Leaching Amount in Water Filtered through Ceramic Water Filter

Ceramic Water Filter	Silver Elution Content (mg/L)
Raw Water	ND
Ceramic water filter (Only $AgNO_3$)	ND
Ceramic water filter ($AgNO_3$ + CS)	0.0143
Ceramic water filter ($AgNO_3$ + SiAg) (0.012 g)	ND
Ceramic water filter ($AgNO_3$+$SiAg_2$) (0.069 g)	ND

The flow rate of the water through the treated and untreated ceramic water filter was measured. It was observed that due to the presence of impurities in the filtered water, the flow rate decreased over time. It was found that in order to maintain the water filtration efficiency, it is necessary to wash the ceramic water filter once a day.

For the experiment, the presence of bacterial contamination in Hlaing River water, Ngamoeyeik creek water, and Hlaw gar tap water was tested using Petrifilm to determine whether bacteria were present before and after filtration through the ceramic water filter coated with SiAg. Blue colonies were reported as *E.coli* bacteria and the sum of the red with bubble and blue colonies were reported as total coliform bacteria. The total count of *E. coli* and Coliform Bacteria is collectively referred to as the Total Coliform count. The results obtained from the Petrifilm tests are presented in Table (4). *E. coli* and Coliform Bacteria were found in high quantities in the Hlaing River water and Ngamoeyeik Creek water. After filtering water through the ceramic water filter coating with SiAg, *E. coli* and Coliform Bacteria were not detected. The Petrifilm test results are shown in Figure (9).

Table 4. Total Coliform Count After passing ceramic water filter coating with SiAg (0.012 g), (0.069 g) and Colloidal Silver

Ceramic Water Filter	Tested Water from Hlaing River		Tested Water from Ngamoeyeik Crrek		Tested Water from Hlawgar Tap Water	
	E. coli	Total Coliform	*E. coli*	Total Coliform	*E. coli*	Total Coliform
Raw Water (RW)	12	58	76	189	ND	15
Ceramic water filter	1	9	5	13	ND	19
Ceramic water filter coated with colloidal silver (CS)	ND	ND	3	9	ND	ND
Ceramic water filter coated with SiAg (0.012 g) (SiAg)	ND	ND	ND	ND	ND	ND
Ceramic water filter coated with SiAg (0.069 g)	ND	ND	ND	ND	ND	ND

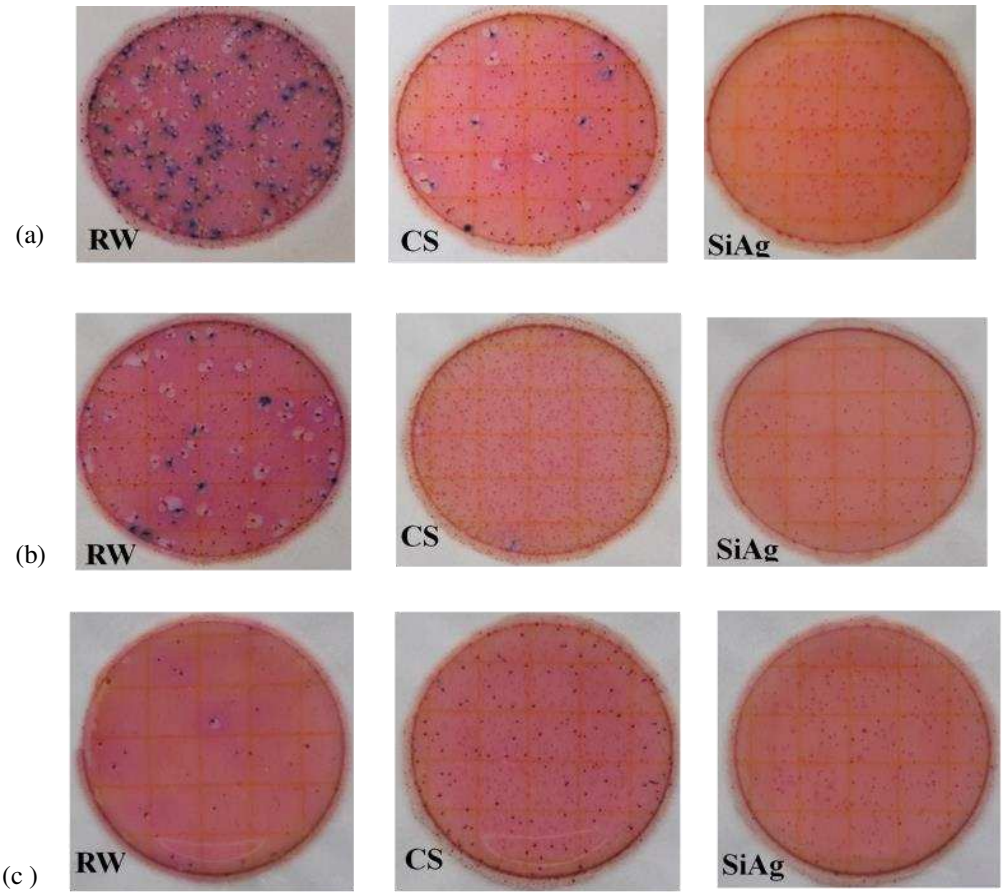

Fig. 8. *E.coli*/ Total Coliform petriflim showing antibacterial activity of ceramic water filter coating with SiAg (0.012 g) and Colloidal Silver (a) water sample from Ngamoeyeik Creek, (b) water sample from Hlaing River and (c) water sample from Hlaing tap water [RW = total coliform count of water sample before passing through the ceramic water filter]

4. Conclusion

SiAg nanocomposites with content of 528 mg/kg were successfully synthesized. According to EDXRF and AAS results, silver ions were found in the composite sample. From UV-vis spectroscopy, AgNPs were uniformly distributed at 408 nm absorption peak. The obtained SiAg product has been used for production of ceramic water filters with effectively bactericidal activity. At silver elution, the silver content was non-detected in filtrated water. These are safe from toxic effect of silver. Silica-silver nanocomposite inhibited the growth of *E. coli* and zone of inhibition ranging from 12 -14 mm at >2.0 mg. The test tube and zone of inhibition tests for *E. coli* prove that the composite possesses a strong antimicrobial activity. Results of bacterial removal in filtered water samples, the percent reduction of total coliform bacteria was 95% and E. coli bacteria was 98%. This study concludes ceramic water filters after silica-silver nanocomposite coating can be used at household level for treating drinking waters especially eliminating microbial contamination. These results all suggest that this technology can effectively improve water quality in the developing world.

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